Remarks

In the Office Action of May 19, 2004, the Examiner rejected the pending claims under various references, claiming that applicants were not able to rely on the section 119 priority date because a certified translation of the priority document had not been filed. As shown by the previous amendment filed in this case, applicant had intended to file a certified translation of the German priority document, DE 199 21 877.0. For some reason, the Examiner did not receive the original certified translation. Enclosed are a copy of the certified translation and a copy of the cover letter from the translation agency. The undersigned attorney apologizes for any error he may have made in sending the documents to the Examiner.

The copy of the certified translation should allow the applicant to claim the priority date of May 12, 1999 and so avoid the von Samson-Himmelstjerna and Nicolay et al. references.

The Examiner also rejected claim 9 under section 112 because of improper dependency. That typographical error has been amended in the present amendment.

Withdrawal of the rejections and allowance of the claims is respectfully requested.

Respectfully Submitted,

Richard S. Bullitt
Reg. No. 30,733

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16 January 2004

Bayer Corp. Morristown
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P.O. Box 1910
Morristown, NJ 07962-1910
U.S.A.

Dear Mr. Richard S. Bullitt

Bayer HealthCare AG Reference: Le A 33 583-FC

In accordance with instructions from Bayer HealthCare AG we are sending you herewith our certified translation of the German Priority document No. 199 21 887.0.

Instructions for filing this case will be sent to you directly from Bayer HealthCare AG, before expiration of the priority date, and may already have been received by you.

Yours faithfully,

pp Sue Anthony, Managing Director
RWS Group plc
UK Translation Division

Enclosure

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ISO 9001 REGISTERED FIRM

UNITED STATES PATENT AND TRADEMARK OFFICE

I, Susan ANTHONY BA, ACIS,

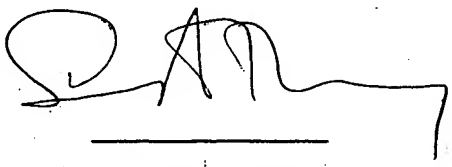
Director of RWS Group plc, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare;

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland.

2. That the translator responsible for the attached translation is well acquainted with the German and English languages.

3. That the attached is, to the best of RWS Group plc knowledge and belief, a true translation into the English language of the accompanying copy of the specification filed with the application for a patent in Germany on 12 May 1999 under the number 199 21 887.0 and the official certificate attached hereto.

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application in the United States of America or any patent issuing thereon.



For and on behalf of RWS Group plc

The 16th day of January 2004

FEDERAL REPUBLIC OF GERMANY

Certificate

Bayer Aktiengesellschaft

of

Leverkusen/Germany

have filed a Patent Application under the title:

"Endoparasiticidal compositions"

on 12 May 1999 at the German Patent and Trademark Office.

The attached document is a correct and accurate reproduction of the original submission for this Patent Application.

The German Patent and Trademark Office has for the time being given the Application the symbol A 01 N 38/15 of the International Patent Classification.

Munich, 10 April 2000

German Patent and Trademark Office

The President

pp

Agurks

File No: 199 21 887.0

Endoparasiticidal compositions

The present invention relates to the use of piperazines for increasing the endoparasiticidal action of cyclic depsipeptides in endoparasiticidal compositions.

Piperazines and their action against endoparasites are generally known. (Mehlhorn et al., Diagnostik und Therapie der Parasitosen des Menschen [Diagnosis and Therapy of Human Parasitoses], 2nd Edition, Gustav Fischer Verlag, (1995), Mehlhorn et al., Diagnostik und Therapie der Parasitosen von Haus-, Nutz- und Heimtieren [Diagnosis and Therapy of Parasitoses of Domestic, Agricultural and Pet Animals], 2nd Edition, Gustav Fischer Verlag, (1993)).

A cyclic depsipeptide PF 1022 and its action against endoparasites is disclosed in EP-OS (German Published Specification) 382 173.

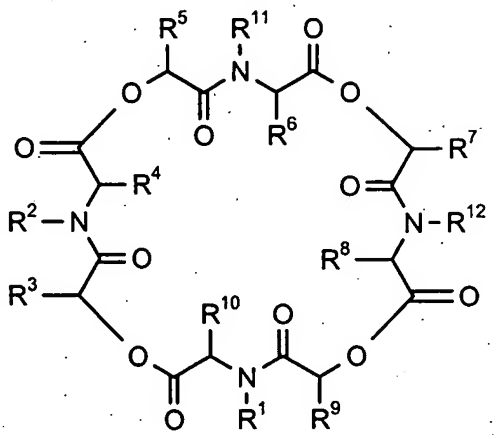
Further cyclic depsipeptides and their endoparasiticidal action are the subject of EP-OS (German Published Specification) 0 626 375, EP-OS (German Published Specification) 0 626 376 and WO 93/25543.

The present invention relates to the use of piperazines for increasing the endoparasiticidal action of cyclic depsipeptides consisting of amino acids and hydroxycarboxylic acids as ring units and [lacuna] 24 ring atoms.

The present invention further relates to endoparasiticidal compositions which contain piperazines together with cyclic depsipeptides consisting of amino acids and hydrocarboxylic acids as ring units and [lacuna] 24 ring atoms.

The present invention further relates to the use of piperazines together with cyclic depsipeptides consisting of amino acids and hydroxycarboxylic acids as ring units and [lacuna] 24 ring atoms for the production of endoparasiticidal compositions.

The cyclic depsipeptides having 24 ring atoms include compounds of the general formula (I)



(I)

in which

R^1, R^2, R^{11} and R^{12} independently of one another represent C_{1-8}-alkyl, C_{1-8}-halogenoalkyl, C_{3-6}-cycloalkyl, aralkyl, aryl,

R^3, R^5, R^7, R^9 independently of one another represents hydrogen or straight-chain or branched C_{1-8}-alkyl, which can optionally be substituted by hydroxyl, C_{1-4}-alkoxy, carboxyl,  , carboxamide,  , imidazolyl, indolyl,

guanidino, -SH or C_{1-4}-alkylthio and further represents aryl or aralkyl which can be substituted by halogen, hydroxyl, C_{1-4}-alkyl, C_{1-4}-alkoxy,

R^4, R^6, R^8, R^{10} independently of one another represent hydrogen, straight-chain C_{1-5}-alkyl, C_{2-6}-alkenyl, C_{3-7}-cycloalkyl, each of which can optionally be substituted by hydroxyl, C_{1-4}-alkoxy, carboxyl, carboxamide, imidazolyl, indolyl, guanidino, SH or C_{1-4}-alkylthio, and represent aryl or aralkyl which can be substituted by halogen, hydroxyl, C_{1-4}-alkyl, C_{1-4}-alkoxy,

and their optical isomers and racemates.

Preferably, compounds of the formula (I) are employed in which

R^1, R^2, R^{11} and R^{12} independently of one another represent methyl, ethyl, propyl, isopropyl, n-, s-, t-butyl or phenyl, which is optionally substituted by halogen, C_{1-4}-alkyl, OH, C_{1-4}-alkoxy, and also represent benzyl or phenylethyl, each of which can optionally be substituted by the radicals indicated in the case of phenyl, and

R^3 to R^{10} have the meaning indicated above.

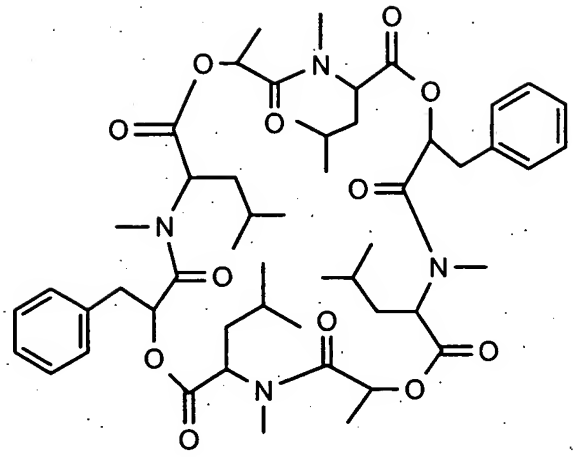
Particularly preferred compounds of the formula (I) are those in which

R^1, R^2, R^{11} and R^{12} independently of one another represent methyl, ethyl, propyl, isopropyl or n-, s-, t-butyl,

R^3, R^5, R^7, R^9 represent hydrogen, straight-chain or branched C_{1-8}-alkyl, in particular methyl, ethyl, propyl, i-propyl, n-, s-, t-butyl, each of which can optionally be substituted by C_{1-4}-alkoxy, in particular methoxy, ethoxy, imidazolyl, indolyl or C_{1-4}-alkylthio, in particular methylthio, ethylthio, and further respresent phenyl, benzyl or phenethyl, each of which can optionally be substituted by halogen, in particular chlorine, and

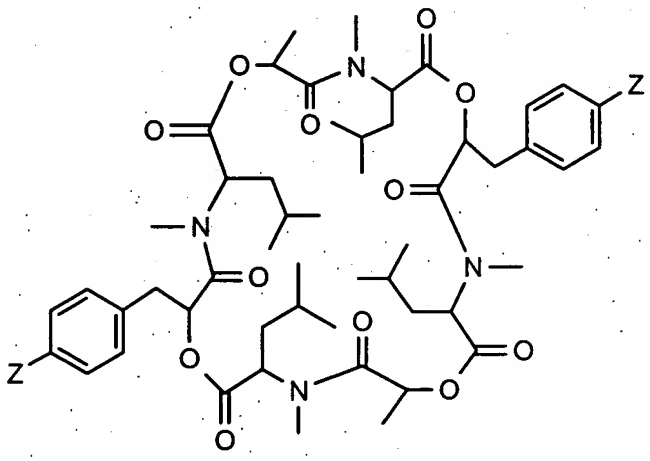
R^4, R^6, R^8, R^{10} independently of one another represent hydrogen, methyl, ethyl, n-propyl, n-butyl, vinyl, cyclohexyl, each of which can optionally be substituted by methoxy, ethoxy, imidazolyl, indolyl, methylthio, ethylthio, and represent isopropyl, s-butyl and further represent optionally halogen-substituted phenyl, benzyl or phenylethyl.

Furthermore, the compound PF 1022 of the following formula disclosed in EP-OS (German Published Specification) 382 173 may be mentioned as a 24 ring-membered depsipeptide:



Moreover, the compounds disclosed in the PCT application WO 93/19053 may be mentioned as depsipeptides.

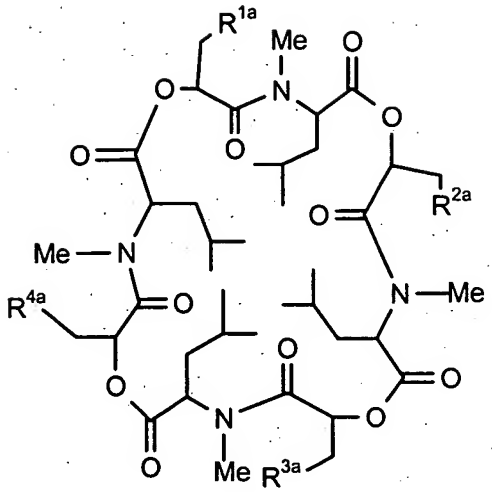
In particular, the compounds of the following formula may be mentioned from PCT application WO 93/19053:



in which

Z represents N-morpholinyl, nitro, amino, mono- or dimethylamino, particularly emphatically N-morpholinyl.

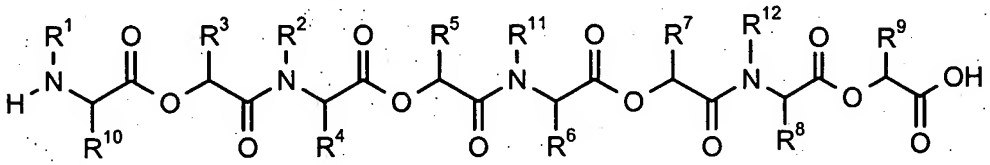
Moreover, compounds of the following formula may be mentioned:



in which

R^{1a}, R^{2a}, R^{3a}, R^{4a} independently of one another represent hydrogen, C_1-C_{10}-alkyl or aryl, in particular phenyl, each of which is optionally substituted by hydroxyl, C_1-C_{10}-alkoxy or halogen.

The compounds of the formula (I) can be prepared by cyclizing open-chain octadepsipeptides of the formula (II)



(II)

in which

R^1 to R^{12} have the meaning indicated above,

in the presence of a diluent and in the presence of a coupling reagent.

Suitable coupling reagents are all compounds which are suitable for the formation of an amide bond (cf., for example: Houben-Weyl, Methoden der organischen Chemie

[Methods of Organic Chemistry], Volume 15/2; Bodanszky et al., Peptide Synthesis 2nd ed. (Wiley / Sons, New York 1976).

The following reagents and methods are preferably suitable: active ester method using pentafluorophenol (Pfp), N-hydroxysuccinimide, 1-hydroxybenzotriazole, coupling using carbodiimides, such as dicyclohexylcarbodiimide or N'-(3-dimethyl-aminopropyl)-N-ethyl-carbodiimide (Ebc), and the mixed anhydride method or coupling using phosphonium reagents, such as benzotriazol-1-yl-oxy-tris(dimethyl-aminophosphonium) hexafluorophosphate (BOP), bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (BOP-Cl), or using phosphonic acid ester reagents, such as diethyl cyanophosphonate (DEPC) and diphenylphospharyl azide (DPPA).

Coupling using bis(2-oxo-3-oxazolidinyl)-phosphonium acid chloride (BOP-Cl) and N'-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC) in the presence of 1-hydroxybenzotriazole (HOBt) is particularly preferred.

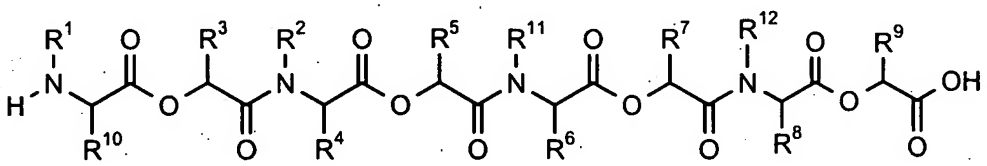
The reaction is carried out at temperatures from 0 – 150°C, preferably at 20 to 100°C, particularly preferably at room temperature.

Suitable diluents are all inert organic solvents. These include, in particular, aliphatic and aromatic, optionally halogenated hydrocarbons, such as pentane, hexane, heptane, cyclohexane, petroleum ether, benzine, ligroin, benzene, toluene, methylene chloride, ethylene chloride, chloroform, carbon tetrachloride, chlorobenzene and o-dichlorobenzene, furthermore ethers such as diethyl and dibutyl ether, glycol dimethyl ether and diglycol dimethyl ether, tetrahydrofuran and dioxane, furthermore ketones, such as acetone, methyl ethyl ketone, methyl isopropyl ketone and methyl isobutyl ketone, moreover esters, such as methyl acetate and ethyl acetate, furthermore nitriles, e.g. acetonitrile and propionitrile, benzonitrile, glutaronitrile, moreover amides, e.g. dimethylformamide, dimethylacetamide and N-methylpyrrolidone, and also dimethyl sulfoxide, tetramethylene sulfone and hexamethylphosphoramide.

The compounds of the formulae (II) and the coupling reagents are employed in the ratio 1:1 to 1:1.5 to one another. An approximately equimolar ratio is preferred.

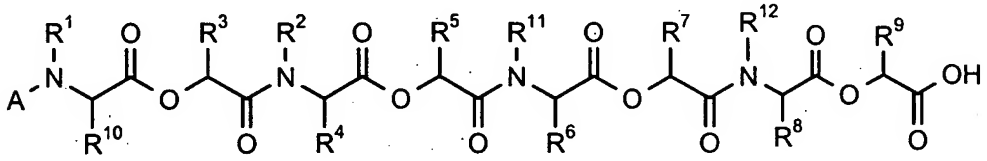
After the reaction has taken place, the diluent is distilled off and the compounds of the formula (I) are purified in a customary manner, e.g. by chromatography.

The open-chain octadepsipeptides of the formula (II)



(II)

in which the radicals have the meanings indicated above are obtained by hydrogenolyzing compounds of the formula (III)



(III)

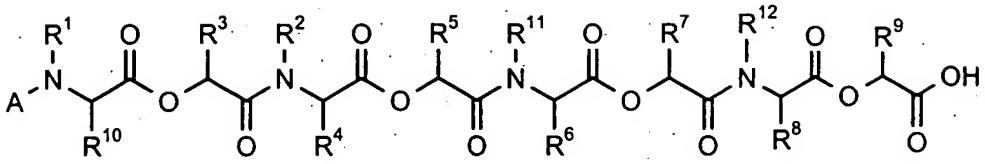
in which

A [lacuna] benzyl and

R^1 to R^{12} have the meaning indicated above,

in the presence of a diluent and of a catalyst.

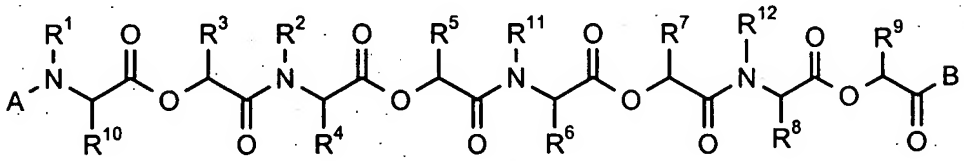
The compounds of the formula (III)



(III)

in which the radicals have the meaning indicated above, are obtained by

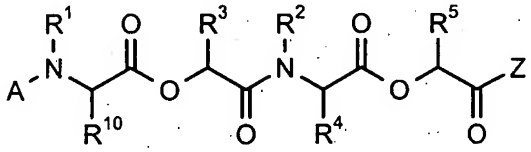
hydrolyzing compounds of the formula (IV)



(IV)

in which the radicals A and R^1 to R^{12} have the meaning indicated above and B represents t-butoxy.

Compounds of the formula (IV) and their stereoisomers are obtained by condensing tetradepsipeptides of the formula (V)



(V)

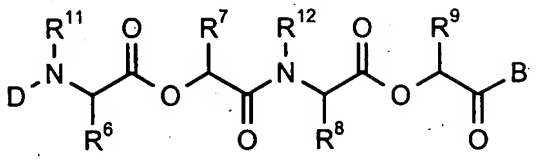
in which

A represents benzyl and

Z represents OH, and

R^1, R^2, R^3, R^4, R^5 and R^{10} have the meaning indicated above

and tetradepsipeptides of the formula (VI)



(VI)

in which

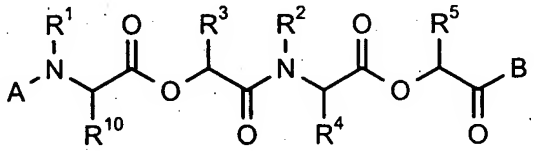
D represents hydrogen and

B represents tert-butoxy, and

R^6, R^7, R^8, R^9, R^{11} and R^{12} have the meaning indicated above,

in the presence of a diluent and of a coupling reagent.

Tetradepsipeptides of the formula (V) are obtained by saponifying tetradepsipeptides of the formula (VII)



(VII)

in which

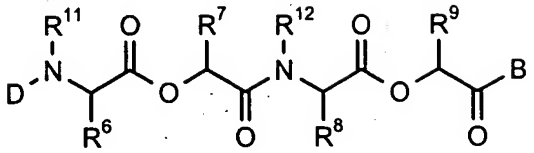
A represents benzyl and

B represents tert-butoxy, and

R^1, R^2, R^3, R^4, R^5 and R^{10} have the meaning indicated above,

in the presence of a diluent and of a protonic acid.

Tetradepsipeptides of the formula (VI)



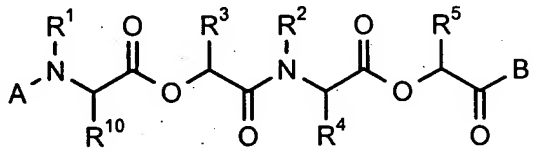
(VI)

in which

D represents hydrogen and

B represents tert-butoxy and the other radicals have the meaning indicated above,

are obtained by hydrogenolyzing tetradepsipeptides of the formula (VII)



(VII)

in which

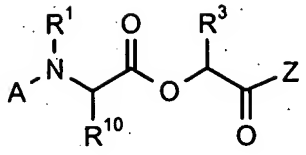
A represents benzyl and

B represents tert-butoxy, and

R^1, R^2, R^3, R^4, R^5 and R^{10} have the meaning indicated above,

in the presence of a diluent and of a catalyst.

Tetradepsipeptides of the formula (VII) are obtained by condensing didepsipeptides of the formula (VIII)



(VIII)

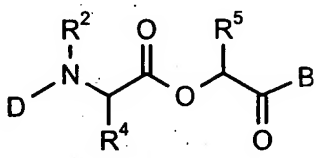
in which

A represents benzyl and

Z represents OH, and

R^1, R^3 and R^{10} have the meaning indicated above and

didepsipeptides of the formula (IX)



(IX)

in which

D represents hydrogen and

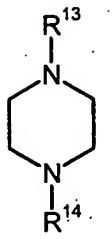
B represents tert-butoxy, and

R^2, R^4 and R^5 have the meaning indicated above,

in a diluent in the presence of a coupling reagent.

The depsipeptides disclosed in WO 93/19 053 or in EP-OS (German Published Specification) 382 173 can be contained by the methods described there.

The piperazines include all compounds of the formula (X)



(X)

in which

R^{13} and R^{14} independently of one another represent identical or different substituents of the group hydrogen, in each case optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, and $-CONR^{15}R^{16}$ or $-CSNR^{15}R^{16}$, in which

R^{15} and R^{16} independently of one another represent identical or different substituents of the group hydrogen, in each case optionally substituted alkyl or cycloalkyl.

Preferred compounds of the formula (X) are those in which

R^{13} and R^{14} independently of one another represent identical or different substituents of the group hydrogen, in each case optionally substituted C_1-C_6-alkyl, C_3-C_8-cycloalkyl, and $-CONR^{15}R^{16}$ or $-CSNR^{15}R^{16}$, in which

R^{15} and R^{16} independently of one another represent identical or different substituents of the group hydrogen, in each case optionally substituted C_1-C_6-alkyl or C_3-C_8-cycloalkyl.

Particularly preferred compounds of the formula (X) are those in which

R^{13} and R^{14} independently of one another represent identical or different substituents of the group hydrogen, in each case optionally

substituted C_1-C_4-alkyl, C_6-cycloalkyl, and -$CONR^{15}R^{16}$ or -$CSNR^{15}R^{16}$, in which

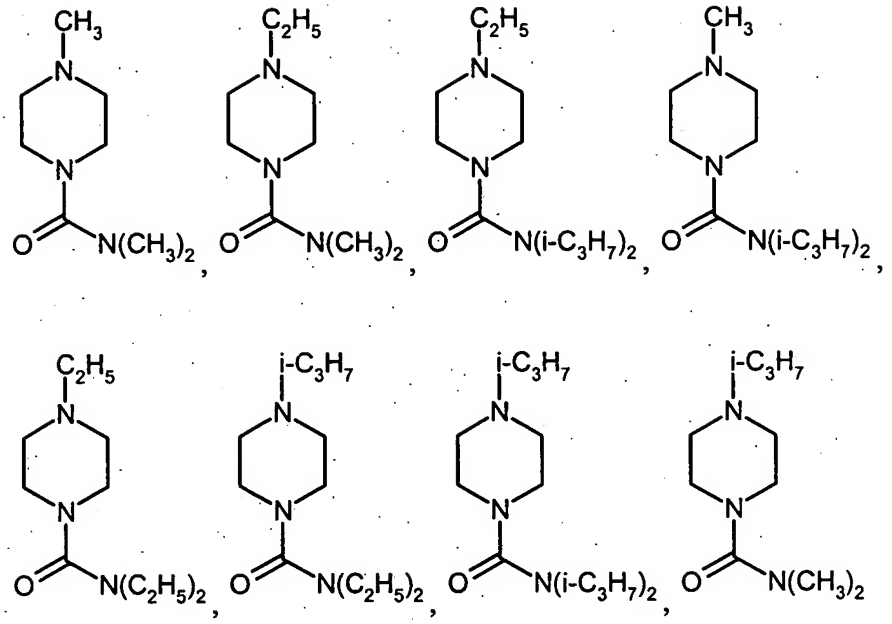
R^{15} and R^{16} independently of one another represent identical or different substituents of the group hydrogen, in each case optionally substituted C_1-C_4-alkyl or C_6-cycloalkyl.

Very particularly preferred compounds of the formula (X) are those in which

R^{13} and R^{14}	independently of one another represent identical or different substituents of the group hydrogen, C_1-C_4-alkyl, C_1-C_4-haloalkyl having 1 to 9 identical or different halogen atoms of the series F, Cl, or Br, C_6-cycloalkyl, and -$CONR^{15}R^{16}$ or -$CSNR^{15}R^{16}$, in which
	R^{15} and R^{16} independently of one another represent identical or different substituents of the group hydrogen, C_1-C_4-alkyl or C_6-cycloalkyl.

The following compounds may be mentioned by way of example, but not restrictively:

piperazine, diethylcarbamazine, N,N′-dimethylpiperazine, N-methylpiperazine, N,N′-diethylpiperazine, N-ethylpiperazine, N-ethyl-N′-methylpiperazine, N,N′-dipropylpiperazine, N-propylpiperazine, N-ethyl-N′-propylpiperazine, N-methyl-N′-propylpiperazine, N-cyclohexylpiperazine, N,N′-dicyclohexylpiperazine,



piperazine and diethylcarbamazine may be especially emphasized here.

The piperazines are generally known organic compounds and are commercially obtainable or can be obtained by known methods. (Mehlhorn et al. Diagnostik und Therapie der Parasitosen des Menschen 2nd Edition, Gustav Fischer (1995), Mehlhorn et al. Diagnostik und Therapie der Parasitosen von Haus-, Nutz- und Heimtieren, 2nd Edition Gustav Fischer (1993)).

The compositions according to the invention are suitable for controlling pathogenic endoparasites which occur in humans and in animal keeping and animal breeding in the case of agricultural animals, breeding animals, zoo animals, laboratory animals, experimental animals and pets and have favorable toxicity to warm-blooded animals. They are effective against all or individual stages of development of the pests and also against resistant and normally sensitive species. As a result of the control of the pathogenic endoparasites, disease, cases of death and yield reductions (e.g. in the production of meat, milk, wool, hides, eggs, honey etc.) should be reduced, so that more economical and simpler animal keeping is possible as a result of the use of the active compounds. The pathogenic endoparasites include cestodes, trematodes, nematodes, acantocephalae, in particular:

From the order of the Pseudophyllidea e.g.: Diphyllobothrium spp., Spirometra spp., Schistocephalus spp., Ligula spp., Bothridium spp., Diphlogonoporus spp.

From the order of the Cyclophyllidea e.g.: Mesocestoides spp., Anoplocephala spp., Paranoplocephala spp., Moniezia spp., Thysanosomsa spp., Thysaniezia spp., Avitellina spp., Stilesia spp., Cittotaenia spp., Andyra spp., Bertiella spp., Taenia spp., Echinococcus spp., Hydatigera spp., Davainea spp., Raillietina spp., Hymenolepis spp., Echinolepis spp., Echinocotyle spp., Diorchis spp., Dipylidium spp., Joyeuxiella spp., Diplopylidium spp.

From the subclass of the Monogenea e.g.: Gyrodactylus spp., Dactylogyrus spp., Polystoma spp.

From the subclass of the Digenea e.g.: Diplostomum spp., Posthodiplostomum spp., Schistosoma spp., Trichobilharzia spp., Ornithobilharzia spp., Austrobilharzia spp., Gigantobilharzia spp., Leucochloridium spp., Brachylaima spp., Echinostoma spp., Echinoparyphium spp., Echinochasmus spp., Hypoderaeum spp., Fasciola spp., Fasciolides spp., Fasciolopsis spp., Cyclocoelum spp., Typhlocoelum spp., Paramphistomum spp., Calicophoron spp., Cotylophoron spp., Gigantocotyle spp., Fischoederius spp., Gastrothylacus spp., Notocotylus spp., Catatropis spp., Plagiorchis spp., Prosthogonimus spp., Dicrocoelium spp., Eurytrema spp., Troglotrema spp., Paragonimus spp., Collyriclum spp., Nanophyetus spp., Opisthorchis spp., Clonorchis spp., Metorchis spp., Heterophyes spp., Metagonimus spp.

From the order of the Enoplida e.g.: Trichuris spp., Capillaria spp., Trichomosoides spp., Trichinella spp.

From the order of Rhabditia e.g.: Micronema spp., Strongyloides spp.

From the order of Strongylida e.g.: Stronylus spp., Triodontophorus spp., Oesophagodontus spp., Trichonema spp., Gyalocephalus spp., Cylindropharynx spp., Poteriostomum spp., Cyclococercus spp., Cylicostephanus spp., Oesophagostomum

spp., Chabertia spp., Stephanurus spp., Ancylostoma spp., Uncinaria spp., Bunostomum spp.

Globocephalus spp., Syngamus spp., Cyathostomum spp., Metastrongylus spp., Dictyocaulus spp., Muellerius spp., Protostrongylus spp., Neostrongylus spp., Cystocaulus spp., Pneumostrongylus spp., Spicocaulus spp., Elaphostrongylus spp., Parelaphostrongylus spp., Crenosoma spp., Paracrenosoma spp., Angiostrongylus spp., Aelurostrongylus spp., Filaroides spp., Parafilaroides spp., Trichostrongylus spp., Haemonchus spp., Ostertagia spp., Marshallagia spp., Cooperia spp., Nematodirus spp., Hyostrongylus spp., Obeliscoides spp., Amidostomum spp., Ollulanus spp., Cylicocyclus spp., Crateostonum spp., Cylicodontophorus spp.

From the order of the Oxyurida e.g.: Oxyuris spp., Enterobius spp., Passalurus spp., Syphacia spp., Aspiculuris spp., Heterakis spp.

From the order of the Ascaridia e.g.: Ascaris spp., Toxascaris spp., Toxocara spp., Parascaris spp., Anisakis spp., Ascaridia spp.

From the order of the Spirurida e.g.: Gnathostoma spp., Physaloptera spp., Thelazia spp., Gongylonema spp., Habronema spp., Parabronema spp., Draschia spp., Dracunculus spp.

From the order of the Filariida e.g.: Stephanofilaria spp., Parafilaria spp., Setaria spp., Loa spp., Dirofilaria spp., Litomosoides spp., Brugia spp., Wuchereria spp., Onchocerca spp.

From the order of the Gigantorhynchida e.g.: Filicollis spp., Moniliformis spp., Macracanthorhynchus spp., Prosthenorchis spp.

The agricultural and breeding animals include mammals such as cattle, horses, sheep, pigs, goats, camels, water buffalo, donkeys, rabbits, fallow deer, reindeer, fur-bearing animals such as mink, chinchilla, racoon, birds such as chickens, geese, turkeys,

ducks ostriches, freshwater and saltwater fish such as trout, carps, eels, reptiles, insects such as honeybees and silkworms.

The laboratory and experimental animals include mice, rats, guinea-pigs, golden hamsters, dogs and cats.

The pets include dogs and cats.

Administration can be carried out both prophylactically and therapeutically.

The active compound mixtures are administered directly or enterally, parenterally, dermally, nasally in the form of suitable preparations, by treatment of the habitat or with the aid of active compound-containing molded articles such as strips, plates, tapes, collars, ear tags, limb bands, marking devices.

Enteral administration of the active compound mixtures is carried out, for example, orally in the form of powders, tablets, capsules, pastes, drinks, granules, orally administrable solutions, suspensions and emulsions, boli, medicated feed or drinking water. Dermal administration is carried out, for example, in the form of dipping, spraying or pouring-on and spotting-on. Parenteral administration is carried out, for example, in the form of injection (intramuscular, subcutaneous, intravenous, intraperitoneal) or by implants.

Suitable preparations are:

Solutions such as injection solutions, oral solutions, concentrates for oral administration after dilution, solutions for use on the skin or in body cavities, pour-on formulations, gels;

Emulsions and suspensions for oral or dermal administration and also for injection; semi-solid preparations;

Formulations in which the active compound mixture is processed in an ointment base or in an oil-in-water or water-in-oil emulsion base;

Solid preparations such as powders, premixes or concentrates, granules, pellets, tablets, boli, capsules; aerosols and inhalants, active compound mixture-containing molded articles.

Injection solutions are administered intravenously, intramuscularly and subcutaneously.

Injection solutions are prepared by dissolving the active compound mixture in a suitable solvent and adding possible additives such as solubilizers, acids, bases, buffer salts, antioxidants, preservatives. The solutions are sterile-filtered and bottled.

Solvents which may be mentioned are: physiologically tolerable solvents such as water, alcohols such as ethanol, butanol, benzyl alcohol, glycerol, propylene glycol, polyethylene glycols, N-methyl-pyrrolidone, and mixtures thereof.

The active compound mixture can optionally also be dissolved in physiologically tolerable vegetable or synthetic oils which are suitable for injection.

Solubilizers which may be mentioned are: solvents which promote the dissolution of the active compound mixture in the main solvent or prevent its precipitation. Examples are polyvinylpyrrolidone, polyoxyethylated castor oil, polyoxyethylated sorbitan ester.

Preservatives are: benzyl alcohol, trichlorobutanol, p-hydroxybenzoic acid ester, n-butanol.

Oral solutions are administered directly. Concentrates are administred orally after prior dilution to the administration concentration. Oral solutions and concentrates are

prepared as described above in the case of the injection solutions, it being possible to dispense with working under sterile conditions.

Solutions for use on the skin are applied drop by drop, smoothed on, rubbed in, splashed on or sprayed on. These solutions are prepared as described above in the case of the injection solutions.

It may be advantageous to add thickeners during the preparation. Thickeners are: inorganic thickeners such bentonites, colloidal silicic acid, aluminum monostearate, organic thickeners such as cellulose derivatives, polyvinyl alcohols and their copolymers, acrylates and metacrylates.

Gels are applied to the skin or smoothed on or introduced into body cavities. Gels are prepared by mixing solutions which have been prepared as described in the case of the injection solutions with sufficient thickener that a clear material having an ointment-like consistency results. The thickeners employed are those indicated further above.

Pour-on formulations are poured onto or splashed onto restricted areas of the skin, the active compound penetrating the skin and acting systemically.

Pour-on formulations are prepared by dissolving, suspending or emulsifying the active compound mixture in suitable skin-compatible solvents or solvent mixtures. If appropriate, further auxiliaries, such as colorants, absorption-promoting substances, antioxidants, light screens, tackifiers are added.

Solvents which may be mentioned are: water, alkanols, glycols, polyethylene glycols, polypropylene glycols, glycerol, aromatic alcohols such as benzyl alcohol, phenyl-ethanol, phenoxyethanol, esters such as ethyl acetate, butyl acetate, benzyl benzoate, ethers such as alkylene glycol alkyl ethers such as dipropylene glycol monomethyl ether, diethylene glycol mono-butyl ether, ketones such as acetone, methyl ethyl ketone, aromatic and/or aliphatic hydrocarbons, vegetable or synthetic oils, DMF,

dimethylacetamide, N-methylpyrrolidone, 2,2-dimethyl-4-oxy-methylene-1,3-dioxolane.

Colorants are all colorants, which can be dissolved or suspended, permitted for use on animals.

Absorption-promoting substances are, for example, DMSO, spreading oils such as isopropyl myristate, dipropylene glycol pelargonate, silicone oils, fatty acid esters, triglycerides, fatty alcohols.

Antioxidants are sulfites or metabisulfites such as potassium metabisulfite, ascorbic acid, butylhydroxytoluene, butylhydroxyanisole, tocopherol.

Light screens are, for example, novantisolic acid.

Tackifiers are, for example, cellulose derivatives, starch derivatives, polyacrylates, natural polymers such as alginates, gelatin.

Emulsions can be administered orally, dermally or as injections.

Emulsions are either of the water-in-oil type or of the oil-in-water type.

They are prepared by dissolving the active compound mixture either in the hydrophobic or in the hydrophilic phase and homogenizing this with the solvent of the other phase with the aid of suitable emulsifiers and, if appropriate, further auxiliaries such as colorants, absorption-promoting substances, preservatives, antioxidants, light screens, viscosity-increasing substances.

Hydrophobic phases (oils) which may be mentioned are: paraffin oils, silicone oils, natural vegetable oils such as sesame oil, almond oil, castor oil, synthetic triglycerides such as caprylic/capric biglyceride, triglyceride mixture with plant fatty acids of chain length C_{8-12} or other specially selected natural fatty acids, partial

glyceride mixtures of saturated or unsaturated fatty acids which possibly also contain hydroxyl groups, mono- and diglycerides of the C_8/C_{10} fatty acids.

Fatty acid esters such as ethyl stearate, di-n-butyryl adipate, hexyl laurate, dipropylene glycol pelargonate, esters of a branched fatty acid of medium chain length with saturated fatty alcohols of chain length C_{16}-C_{18}, isopropyl myristate, isopropyl palmitate, caprylic/capric acid esters of saturated fatty alcohols of chain length C_{12}-C_{18}, isopropyl stearate, oleyl oleate, decyl oleate, ethyl oleate, ethyl lactate, waxy fatty acid esters such as artificial duck uropygial gland fat, dibutyl phthalate, diisopropyl adipate, ester mixtures related to the latter etc.

Fatty alcohols such as isotridecyl alcohol, 2-octyldodecanol, cetylstearyl alcohol, oleyl alcohol.

Fatty acids such as oleic acid and its mixtures.

Hydrophilic phases which may be mentioned are:

Water, alcohols such as propylene glycol, glycerol, sorbitol and their mixtures.

Emulsifiers which may be mentioned are: nonionic surfactants, e.g. polyoxyethylated castor oil, polyoxyethylenated sorbitan monooleate, sorbitan monostearate, glyceryl monostearate, polyoxyethyl stearate, alkylphenol polyglycol ether;

ampholytic surfactants such as di-Na N-lauryl-ß-iminodipropionate or lecithin;

anionic surfactants, such as Na laurylsulfate, fatty alcohol ether sulfates, mono/dialkyl polyglycol ether orthophosphoric acid ester monoethanolamine salt;

cationic surfactants such as cetyltrimethylammonium chloride.

Further auxiliaries which may be mentioned are: substances which increase viscosity and stabilize the emulsion such as carboxymethylcellulose, methylcellulose and other cellulose and starch derivatives, polyacrylates, alginates, gelatin, gum arabic, polyvinylpyrrolidone, polyvinyl alcohol, copolymers of methyl vinyl ether and maleic anhydride, polyethylene glycols, waxes, colloidal silicic acid or mixtures of the substances mentioned.

Suspensions can be administered orally, dermally or as an injection. They are prepared by suspending the active compound in a carrier liquid, if appropriate with addition of further auxiliaries such as wetting agents, colorants, absorption-promoting substances, preservatives, antioxidants light screens.

Carrier liquids which may be mentioned are all homogeneous solvents and solvent mixtures.

Wetting agents (dispersants) which may be mentioned are the surfactants indicated further above.

Further auxiliaries which may be mentioned are those indicated further above.

Semi-solid preparations can be administered orally or dermally. They differ from the suspensions and emulsions described above only by their higher viscosity.

For the production of solid preparations, the active compound is mixed with suitable carriers, if appropriate with addition of auxiliaries, and brought into the desired form.

Carriers which may be mentioned are all physiologically tolerable solid inert substances. Those which may be used are inorganic and organic substances. Inorganic substances are, for example, sodium chloride, carbonates such as calcium carbonate, hydrogencarbonates, aluminas, silicic acids, argillaceous earths, precipitated or colloidal silica, phosphates.

Organic substances are, for example, sugar, cellulose, foodstuffs and feedstuffs such as milk powder, animal meals, cereal meals and coarse cereal meals, starches.

Auxiliaries are preservatives, antioxidants and colorants which have already been mentioned further above.

Further suitable auxiliaries are lubricants and glidants such as magnesium stearate, stearic acid, talc, bentonites, disintegration-promoting substances such as starch or crosslinked polyvinylpyrrolidone, binders such as starch, gelatin or linear polyvinylpyrrolidone, and dry binders such as microcrystalline cellulose.

In the preparations, the active compound mixtures can also be present as a mixture with further synergists or with other active compounds which act against pathogenic endoparasites. Such active compounds are, for example, L-2,3,5,6-tetrahydro-6-phenyl-imidazothiazole, benzimidazole carbamates, pyrantel.

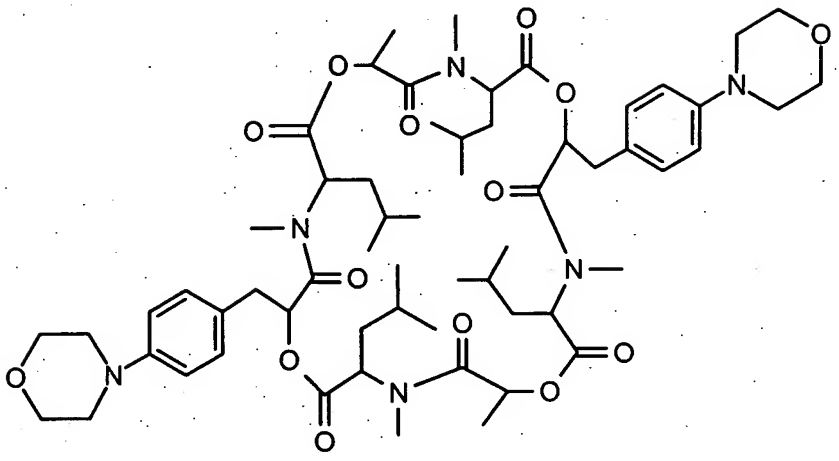
Ready-to-use preparations contain the active compound mixtures in concentrations of 10 ppm – 20% by weight, preferably of 0.1-10% by weight.

Preparations which are diluted before administration contain the active compound mixtures in concentrations of 0.5-90% by weight, preferably of 5 to 50 percent by weight.

In general, it has proven advantageous to administer amounts of the mixture according to the invention of approximately 10 to approximately 100 mg of active compound mixture per kg of body weight per day to achieve effective results. 10 to 50 mg of active compound mixture per kg of body weight are preferred.

In general, a weight ratio of piperazine to depsipeptide such as 50:1 to 1000:1, preferably 100:1 to 1000:1, very particularly preferably 250:1 to 1000:1, in particular 250:1 and 1000:1, is adhered to in the compositions.

In the biological examples, the compound of the formula



disclosed in WO 93/19 053, was employed as "depsipeptide I".

The biological tests were carried out according to the known procedures (Plant et al. Pesticide Science, 1996, 48, p. 351 ff.).

Biological examples

Table 1

Synergistic effect of piperazine and depsipeptide I against Trichinella spiralis in vitro

	Concentration (µg/ml)	Action
Piperazine	1000	0-1
	500	0
Depsipeptide I	0.01	0-1
	0.001	0
Piperazine/depsipeptide I	1000/0.01	1-2
Piperazine/depsipeptide I	500/0.01	1-2

0 = no action; 1 = weak action; 2 = good action

Table 2

Synergistic effect of piperazine and depsipeptide I against mouse nematodes

Heterakis spumosa	Dose (mg/kg)	Action	Nematospiroides dibius	Dose (mg/kg)	Action
Piperazine	4 x 250	1	Piperazine	2 x 2000	2
	4 x 100	0		4 x 1000	1
Depsipeptide I	4 x 1	1	PF1022-221	4 x 1	2
	4 x 0.5	1		4 x 0.5	0-2
Piperazine/ depsipeptide I	4 x 250/ 4 x 1	3	Piperazine/ PF1022-221	2 x 2000/ 4 x 1	2-3
Piperazine/ depsipeptide I	4 x 100/ 4 x 1	2	Piperazine/ PF1022-221	4 x 1000/ 4 x 1	2-3

0 = worm reduction <50 %; 1 = worm reduction 50-75 %; 2 = worm reduction 75-90 %; 3 = complete action, worm reduction >90 %

Preparation examples

Examples of the preparation of the cyclic depsipeptides having 24 ring atoms:

1. Preparation of the compounds of the formula (I).

BOP-Cl (0.124 mmol) was added at 0°C to a solution of the compound of the formula II (0.104 mmol) and Hünig's base (0.258 mmol) in dichloromethane (100 ml) and the mixture was stirred at room temperature for 24 h. After this time, the same amounts of BOP-Cl and base were added and the mixture was stirred for a further 24 h. The solution was washed twice with satd sodium hydrogencarbonate solution, dried over sodium sulfate and concentrated. The residue was purified by column chromatography using the eluent cyclohexane/ethyl acetate 2:1.

Compounds of the formula (I) were obtained in which the substituents have the following meaning (table 3):

Table 3

No.	R^{1a}	R^{2a}	R^{3a}	R^{4a}	R^{5a}	R^{6a}	R^{7a}	R^{8a}	R^{9a}	R^{10a}	R^{11a}	R^{12a}	FAB-MS m/z (%)
1	Et	Et	Me	s-Bu	Bn	s-Bu	Me	s-Bu	Bn	s-Bu	Et	Et	
2	Propyl	Propyl	"	"	"	"	"	"	"	"	Propyl	Propyl	
3	i-Propyl	i-Propyl	"	"	"	"	"	"	"	"	i-Propyl	i-Propyl	
4	Me	Me	"	s-Bu	"	s-Bu	"	s-Bu	"	s-Bu	Me	Me	948 (82, $(+H)^+$)
5	Me	Me	"	i-Pr	"	i-Pr	"	i-Pr	"	i-Pr	Me	Me	915 (100, $(M+Na)^+$) 893 (55, $(M+H)^+$)
6	Me	Me	"	Bn	"	Bn	"	Bn	"	Bn	Me	Me	1107 (100, $(M+Na)^+$) 1085 (8, $(M+H)^+$)
7	Me	Me	"	s-Bu	2-Cl-Bn	s-Bu	"	s-Bu	2-Cl-Bn	s-Bu	Me	Me	
8	Me	Me	"	"	2-Cl-Bn	"	"	"	3-Cl-Bn	"	Me	Me	
9	Me	Me	"	"	4-Cl-Bn	"	"	"	4-Cl-Bn	"	Me	Me	
10	Propyl	i-Propyl	"	"	-Bn	"	"	"	-Bn	"	Propyl	i-Propyl	

Me = methyl
Et = ethyl
Bu = butyl
Pr = propyl
Bn = benzyl

Examples of the preparation of the compounds of the formula (II)

A solution of an open-chain octadepsipeptide of the formula (III) (1.222 mmol) in ethanol (50 ml) was hydrogenated in the presence of $Pd(OH)_2/C$ (20%; 200 mg) until the absorption of hydrogen was complete (about 2 h). After filtering off the catalyst, pure compound of the formula II was obtained, which was reacted further without additional purification.

According to this procedure, compounds of the formula (II) were obtained in which the substituents have the meaning according to table 4.

Table 4

No.	R^{1a}	R^{2a}	R^{3a}	R^{4a}	R^{5a}	R^{6a}	R^{7a}	R^{8a}	R^{9a}	R^{10a}	R^{11a}	R^{12a}
11	Et	Et	Me	s-Bu	Bn	s-Bu	Me	s-Bu	Bn	s-Bu	Et	Et
12	Propyl	Propyl	"	"	"	"	"	"	"	"	Propyl	Propyl
13	i-Propyl	i-Propyl	"	"	"	"	"	"	"	"	i-Propyl	i-Propyl
14	Me	Me	"	"	"	"	"	"	"	"	Me	Me
15	Me	Me	"	i-Pr	"	i-Pr	"	i-Pr	"	i-Pr	Me	Me
16	M4	Me	"	Bn	"	Bn	"	Bn	"	Bu	Me	Me
17	Me	Me	"	s-Bu	2-Cl-Bn	s-Bu	"	s-Bu	2-Cl-Bn	s-Bu	Me	Me
18	Me	Me	"	"	3-Cl-Bn	"	"	"	3-Cl-Bn	"	Me	Me
19	Me	Me	"	"	4-Cl-Bn	"	"	"	4-Cl-Bn	"	Me	Me
20	Propyl	i-Propyl	"	"	-Bn	"	"	"	-Bn	"	Propyl	i-Propyl

Me = methyl

Et = ethyl

s-Bu = s-butyl

Bn = benzyl

Preparation of the compounds of the formula (III)

HCl gas was passed into a solution of the tert-butyl ester of the formula (IV) (1.609 mmol) in dichloromethane (40 ml) at 0°C for 1.5 h. The mixture was then warmed to room temperature and stirred for 12 h. The solution was concentrated in a rotary evaporator and dried in a high vacuum. The residue was reacted without further purification.

Analogously, compounds of the formula (III) were obtained in which the substituents have the following meaning (table 5):

Table 5

No.	R^{1a}	R^{2a}	R^{3a}	R^{4a}	R^{5a}	R^{6a}	R^{7a}	R^{8a}	R^{9a}	R^{10a}	R^{11a}	R^{12a}	A
21	Et	Et	Me	s-Bu	Bn	s-Bu	Me	s-Bu	Bn	s-Bu	Et	Et	Bn
22	Propyl	Propyl	"	"	"	"	"	"	"	"	Propyl	Propyl	"
23	i-Propyl	i-Propyl	"	"	"	"	"	"	"	"	i-Propyl	i-Propyl	"
24	Me	Me	"	"	"	"	"	"	"	"	Me	Me	"
25	Me	Me	"	i-Pr	"	i-Pr	"	i-Pr	"	i-Pr	Me	Me	"
26	M4	Me	"	Bn	"	Bn	"	Bn	"	Bn	Me	Me	"
27	Me	Me	"	s-Bu	2-Cl-Bn	s-Bu	"	s-Bu	2-Cl-Bn	s-Bu	Me	Me	"
28	Me	Me	"	"	2-Cl-Bn	"	"	"	3-Cl-Bn	"	Me	Me	"
29	Me	Me	"	"	4-Cl-Bn	"	"	"	4-Cl-Bn	"	Me	Me	"
30	Propyl	i-Propyl	"	"	-Bn	"	"	"	-Bn	"	Propyl	i-Propyl	"

Me = methyl

Et = ethyl

s-Bu = s-butyl

Bn = benzyl

Preparation of the compounds of the formula (IV)

A solution of ethyldiisopropylamine (0.912 mmol) and BOP-Cl (0.438 mmol) was added at 0°C to a solution of the tetradepsipeptides of the formula (VI) and (V) each (2.52 mmol) in dichloromethane (15 ml). The mixture was stirred at 0°C for 1 h and at room temperature for 1.5 h, diluted with 20 ml of dichloromethane, washed twice with a little water, dried over Na_2SO_4 and concentrated. The residue was purified on silica gel using the eluent cyclohexane/t-BuOMe = 2:1.

Preparation of the compounds of the formula (V)

HCl gas was passed at 0°C for 2 h into a solution of the tetradepsipeptide having the formula (VII) (2.848 mmol) in dichloromethane (50 ml).

The mixture was then stirred at room temperature for 8 h, concentrated and dried in a high vacuum. The residue was employed without further purification.

Preparation of the compounds of the formula (VI)

A solution of the tetradepsipeptide having the formula (VII) (9.53 mmol) in ethanol (37 ml) was treated with $Pd(OH)_2/C$ (20%) (0.6 g) and hydrogenated at room temperature and normal pressure for about 3 h. The reaction mixture was filtered, concentrated and the residue was separated on silica gel using the eluent t-BuOMe/cyclohexane/ethanol = 1:1:0.5.

Preparation of the compounds of the formula (VII)

A solution of the didepsipeptide IX (22.9 mmol) and of the didepsipeptide VIIIa (27.5 mmol) in dichloromethane (80 ml) cooled to 0°C was treated with diisopropylethylamine (57.3 mmol) and BOP-Cl (29.8 mmol), stirred at 0°C for 1 h and at room temperature for 1 h. After filtering off the precipitate, the solution was diluted with dichloromethane, washed three times with a little water, dried over

sodium sulfate and concentrated. The residue was separated on silica gel using the eluent cyclohexane/ethyl acetate = 15:1.

Patent claims

1. The use of piperazines for increasing the endoparasiticidal action of cyclic depsipeptides consisting of amino acids and hydroxycarboxylic acids as ring units and having 24 ring atoms.

2. An endoparasiticidal composition which contains piperazines together with cyclic depsipeptides consisting of amino acids and hydroxycarboxylic acids as ring units and having 24 ring atoms.

3. The use of piperazines together with cyclic depsipeptides consisting of amino acids and hydroxycarboxylic acids as ring units and having 24 ring atoms for the production of endoparasiticidal compositions.

Endoparasiticidal compositions

Abstract

The present invention relates to the use of piperazines for increasing the endoparasiticidal action of cyclic depsipeptides, consisting of amino acids and hydroxycarboxylic acids as ring units and having 24 ring atoms, in endoparasiticidal compositions.